



# C6 Flow Cytometer<sup>®</sup> Instrument Manual

Science is hard. Flow cytometry should be easy.<sup>®</sup>

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## 1. DELIVERY AND INSPECTION

The instrument is tested before shipping. International symbols and special handling instructions are printed on the shipping cartons to inform the carrier of the precautions and care applicable to electronic instruments.

On receipt of the instrument, all cartons should be carefully inspected. If signs of mishandling or damage are seen, a claim should be filed with the carrier immediately. If separately insured, a claim should be filed with the insurer.

### 1.1. Package Contents

CFlow software refers generically to either CFlow<sup>®</sup> or CFlow Plus software.

**Table 1.** Accuri C6 Flow Cytometer System Shipping Contents

<b>Computer (Boxes 1 and 2)</b>	<b>C6 Flow Cytometer (Boxes 3 and 4)</b>
<ul style="list-style-type: none"><li>• Computer (CPU)</li><li>• Monitor</li><li>• Video Cable (monitor)</li><li>• Power Cord (monitor)</li><li>• Power Supply and Cord (computer)</li><li>• USB Keyboard</li><li>• USB Mouse</li><li>• Dell Computer Set Up Instruction Card</li></ul>	<ul style="list-style-type: none"><li>• C6 Flow Cytometer</li><li>• Power Supply and Cord (black)</li><li>• USB Cable</li><li>• C6 Flow Cytometer Instruction Manual</li><li>• CFlow Software CD</li><li>• CFlow Quick Start Guide</li><li>• Fluidics Bottle Tray</li><li>• Fluidics Harness</li><li>• Sheath Bottle, 1L - blue ring</li><li>• Waste Bottle, 1L - red ring</li><li>• Cleaner Bottle, 0.25L - green ring</li><li>• Decontamination Bottle, 0.25L - yellow ring</li><li>• Decontamination Conc. Solution (KR-200) - 1</li><li>• Cleaning Conc. Solution (KR-210) - 1</li><li>• Bacteriostatic Conc. Solution (KR-220) - 1</li><li>• In-line Sheath Filters (CP-140) - 2</li><li>• Fluidic Bottle Filter - Large (CP-130) - 1</li><li>• Fluidic Bottle Filter - Small (CP-135) - 2</li><li>• Validation Beads</li></ul>

### 1.2. Items Not Provided

- 1) Surge Suppressor or Uninterruptible Power Supply (UPS). If a UPS is used with the C6 it is recommended to select a model rated at a minimum of 1000VA, to which the cytometer, computer and monitor should be connected.
- 2) Anti-Virus Software
- 3) Microsoft<sup>®</sup> Office or equivalent

It is the responsibility of the user/buyer to install and maintain anti-virus software on the computer workstation.

## 2. SAFETY

### 2.1. General Warnings

THE C6 FLOW CYTOMETER IS INTENDED FOR GENERAL LABORATORY AND **RESEARCH USE ONLY**. IT IS **NOT** INTENDED FOR *IN VITRO* DIAGNOSTIC TESTING. READ THE ENTIRE INSTRUMENT MANUAL BEFORE ATTEMPTING TO OPERATE INSTRUMENT. DO NOT ATTEMPT TO PERFORM ANY PROCEDURE BEFORE CAREFULLY READING ALL INSTRUCTIONS. ALWAYS FOLLOW PRODUCT LABELING AND MANUFACTURER'S RECOMMENDATIONS. IF THERE IS ANY QUESTION AS TO HOW TO PROCEED, CONTACT ACCURI TECHNICAL SUPPORT AT 1.734.994.8000.

IF THIS INSTRUMENT IS USED IN A MANNER NOT SPECIFIED BY THE MANUFACTURER, THE PROTECTION PROVIDED BY THE INSTRUMENT MAY BE IMPAIRED.

ACCURI CYTOMETERS, INC. URGES ITS CUSTOMERS TO COMPLY WITH ALL NATIONAL HEALTH AND SAFETY STANDARDS SUCH AS THE USE OF BARRIER PROTECTION. THIS MAY INCLUDE, BUT IT IS NOT LIMITED TO, PROTECTIVE EYEWEAR, GLOVES, AND SUITABLE LABORATORY ATTIRE WHEN OPERATING OR MAINTAINING THIS INSTRUMENT.

The information in this manual is subject to change at any time without notice. Accuri Cytometers, Inc. reserves the right to change its products in response to technological advancements.

Below is a list of terms and their definitions as used within this manual to indicate a potential hazard.

<b>WARNING</b>	Can cause injury.
<b>CAUTION</b>	Can cause damage to the instrument.

### 2.2. Operator / User Safety

#### **WARNING**

There is a risk of operator injury if:

- The cover is not opened and/or closed with care.
- All covers and panels are not closed and secured in place prior to and during instrument operation.
- Contact is made with moving parts.
- Broken parts are mishandled.
- Improper tools are used for troubleshooting.
- The integrity of safety interlocks and/or sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.

To avoid injury:

- Open and close cover with care.
- Keep covers and panels closed and secured in place while the instrument is in use.
- Keep away from moving parts.
- Report any broken parts to Accuri Technical Support.
- Use the proper tools when troubleshooting.
- Take full advantage of all safety features and do not defeat safety interlocks and/or sensors.
- Acknowledge and act upon instrument alarms and error messages.

IF THIS INSTRUMENT IS USED IN A MANNER NOT SPECIFIED BY THE MANUFACTURER, THE SAFETY PROTECTION PROVIDED BY THE INSTRUMENT MAY BE COMPROMISED LEADING TO OPERATIONAL FAILURE AND/OR OPERATOR INJURY.

To help ensure compliance with intended use:

- Operate the instrument as described in this Instruction Manual.
- Only operate the Accuri C6 Flow Cytometer with an original copyrighted version of software authorized by Accuri.
- Install, update, and run anti-virus protection software on a regular basis.

If you purchased this product from anyone other than Accuri or an authorized Accuri distributor, and, if it is not presently under an Accuri service contract, Accuri cannot guarantee that the product is fitted with the latest mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. For information, call Accuri Technical Support at 1.734.994.8000.

## 2.3. Safety Symbols and Labels

Safety symbols and labels alert you to potentially dangerous conditions. These symbols, together with text, apply to specific procedures and appear as needed on the instrument and throughout this manual. Do not remove these labels.



**Biohazard / Biological risk.** Consider all materials (specimens, reagents, controls, etc.) and areas these materials come into contact with as being potentially infectious and/or life threatening. Wear appropriate laboratory attire, follow universal laboratory safety protocols, and adhere to local regulations.



**Electrical shock hazard.** There is the possibility of electrical shock when the instrument is plugged into the power source.



**Laser radiation / hazard.** Consider all laser sources as being potentially hazardous to eyesight. Wear the proper protective eyewear to avoid damage to your eyes from the lasers. Never look directly into laser light.



**Laser aperture hazard.** Directed laser light is emitted from the aperture indicated with this label. Follow all laser radiation / hazard warnings.

## 2.4. Biological Handling Precautions



Biological samples are potentially dangerous and/or life threatening. Proper handling procedures for samples and reagents should be adhered to at all times.

Depending on the laboratory environment, there could be a risk of biological, chemical, or radiological contamination if you have contact with samples, sample tubes, sample waste, the waste container, and/or associated tubing. Handle all samples as if potentially infectious or life threatening. Wear protective clothing, gloves, and eyewear. Never pipette by mouth. Clean up all spills immediately. Dispose of all samples and waste according to proper handling procedures and local regulations.

- Always treat waste with 0.5% NaOCl before disposal. Dispose of waste according to local regulations.
- Always empty the waste tank daily or when prompted by CFlow software to prevent spillover and possible biohazard risk.
- Consult appropriate Material Safety Data Sheets when necessary. Refer to Section 2.6.e.

The Accuri C6 Flow Cytometer does **not** produce aerosols during normal operation.

Always protect the Sample Introduction Probe (SIP) with a tube of HPLC reagent grade filtered, deionized H<sub>2</sub>O (0.22 µm filter), or equivalent, when the cytometer is not running samples. The C6 Flow Cytometer is programmed for automatic decontamination of the fluidics system during shut down.

## 2.5. Laser Precautions



The C6 Flow Cytometer contains a solid state 488nm and a 640nm diode laser under the outer and secondary covers. The instrument, therefore, may pose certain hazards associated with these lasers, if misused.

Eye and skin exposure to direct and reflected laser light is hazardous and may be extremely harmful. Never remove or attempt to remove the internal covers.

Ensure that all optical filters are securely positioned. Prevent stray reflections from other surfaces. Never place any foreign object in the path of the laser beam.

Only Accuri personnel can install, remove, or repair the lasers. Do not open the Laser Head enclosures for any reason. Always return the instrument to Accuri for repair.

Never operate the unit in the presence of flammable gases or fumes.

Turn off the cytometer when not in use.

### 2.5.a. Laser Interlock

**WARNING** There is risk of personal injury if the laser safety interlock is bypassed. Do not tamper with the laser interlock.

The laser interlock deactivates the laser when the top cover is lifted up. There is a risk of personal injury if the laser safety interlock is bypassed. Never attempt to override the interlock.

## 2.6. Operational Precautions

### 2.6.a. Ambient Temperature and Humidity

Operating temperature is between 15°C and 30°C (59°F and 86°F) and < 80% relative humidity.

### 2.6.b. Area of Use

The C6 Flow Cytometer is designed to fit on a laboratory bench top. Provide a safety perimeter of six (6) inches around the instrument and computer to allow for proper ventilation and to protect the instrument and computer from accidental liquid spills.

Special care must be taken while handling fluids around the cytometer. Care must be taken that uncontained fluids do not enter the interior of the instrument or computer. Clean up spills immediately. Never place anything on top of the C6, including sample tubes and racks.

Turn off the instrument and unplug the power cord before manual cleaning of the cytometer.

If it becomes necessary to move the instrument, do not cause mechanical shock.

### 2.6.c. Electrical Precautions



Electrical devices pose the risk of an electric shock. To reduce the risk of an electric shock, do not open or remove the top cover of the cytometer while the instrument is turned on unless specifically indicated in this Instrument Manual. Only authorized Accuri personnel should remove any other panels from this device.

Always use the provided power cords, power supplies and cables. Do not abuse the cords. Never use the cords to pull the plug from an outlet. Keep cords away from heat, oil or sharp edges. Damaged cords increase the risk of electric shock.

**CAUTION** This instrument must be plugged into a standard, grounded or earthed mains electrical outlet, conforming to local codes. Non-grounded or non-earthed mains outlet adaptors *must not* be used.

### 2.6.d. Reagents

Only fluids approved for use in the Accuri C6 Flow Cytometer should be used in the operation or cleaning of this instrument. For a list of approved reagents for use with this system, refer to Table 2.

**Table 2.** Approved Reagents

Fluid	Description
Sheath Fluid	0.2 µm filtered and deionized H <sub>2</sub> O (HPLC reagent or Milli-Q® grade) with Bacteriostatic Concentrate Solution (Part# KR-220) added
Cleaning Solution	Cleaning Concentrate Solution (Part# KR-210), 10% solution in filtered, de-ionized H <sub>2</sub> O
Decontamination Solution	Decontamination Concentrate Solution (Part# KR-200), 10% solution in filtered, de-ionized H <sub>2</sub> O

Do not use household bleach as a decontamination solution as it contains fluorescent whitening agents that may interfere with fluorescent staining.

## 2.6.e. Material Safety Data Sheets (MSDS)

To obtain an MSDS:

1. Visit [www.AccuriCytometers.com](http://www.AccuriCytometers.com)
2. Email [TechSupport@AccuriCytometers.com](mailto:TechSupport@AccuriCytometers.com)
3. Call Accuri Technical Support at 1.734.994.8000
4. Fax Accuri Technical Support at 1.734.994.8002
5. Or write to: Accuri Cytometers, Attn: Technical Support, PO Box 1388, Ann Arbor, MI 48106 USA

## 3. C6 FLOW CYTOMETER INTRODUCTION

### 3.1. Overview

The C6 Flow Cytometer System offers an entirely new vision for the role of flow cytometry in life science research. Designed from the ground up to be compact and easy to use, the C6 Flow Cytometer fits in any lab and is readily accessible to all researchers, experts and novices alike.

Accuri accomplishes this by providing leading-edge solutions for the three key elements of a cytometer: fluidics, optics, and electronics.

- Accuri's patented fluidics system pulse dampeners and pressure sensors allow the use of peristaltic pumps to provide a non-pressurized, zero pulsation "push/pull" system with sophisticated microprocessor controlled dynamic feedback.
- Clustered in a "pie" configuration around the flow cell, the C6's detectors maximize light collection, reduce alignment issues, and allow the user to easily change interference filters.
- The sophisticated C6 Flow Cytometer electronics system provides six decades of dynamic range eliminating the need to adjust detector voltage and gain settings.
- The C6 Flow Cytometer is USB compatible with a PC or laptop.

Powerful, robust, compact, and affordable, Accuri's two-laser, six-detector, analytical C6 Flow Cytometer works with many existing reagents and protocols and is about the size of a small water bath, 11.0"H x 14.3"W x 16.5"D (27.9 x 36.3 x 41.9cm).

The C6 Flow Cytometer System includes Accuri's CFlow or CFlow Plus software for controlling the instrument, generating statistics, and analyzing results. Ease-of-use was a top priority, so Accuri partnered with Menlo Innovations™ (Ann Arbor, MI, USA), in consultation with many outside researchers, to ensure a user-focused design and a friendly user interface.

The intuitive and innovative C6 Flow Cytometer and CFlow software allows researchers to be running samples within an hour of receiving the system.



**Figure 1.** The C6 Flow Cytometer System

### 3.2. Intended Use

The C6 Flow Cytometer is intended for general laboratory research applications only, **not** for *in vitro* diagnostic (IVD) testing. If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be impaired and the user put at risk.

### 3.3. C6 Requirements

**Table 3.** General Information about the C6 Flow Cytometer

<b>Power (Cytometer only)</b>	AC Input: 100-240V, 50-60Hz, 1.5A DC Output: +19V, 3.7A & 70W max.
<b>Operating ranges</b>	15-30°C, <80% relative humidity
<b>Instrument size</b>	11.0"H x 14.3"W x 16.5"D (27.9 x 36.3 x 41.9 cm) 27"H x 14.8"W x 17.0"D (68.6 x 37.6 x 43.2 cm) with cover fully open Fluidics bottle tray 10"H x 13.5"W x 4.5"D (25.4 x 34.2 x 11.4 cm)
<b>Weight</b>	< 30 lbs (13.6 kg)
<b>Safety perimeter</b>	6" (15 cm)

### 3.4. C6 Flow Cytometer Specifications

**Table 4.** Accuri C6 Flow Cytometer System Specifications

<b>Excitation</b>	488nm; 50mW solid state (rated at 20,000hr life) 640nm; 30mW diode (rated at 10,000hr life)
<b>Laser profile</b>	15x75 microns
<b>Scatter detection</b>	Forward (0°, +/-15°) Side (90°, +/-15°)
<b>Emission detection</b> (using standard filter set provided)	488 Excitation (Blue laser) FL1: 530±15nm (FITC/GFP) FL2: 585±20nm (PE/PI) FL3: >670nm (PerCP-Cy5.5, PE-Cy5, PE-Cy7) 640 Excitation (Red laser) FL4: 675±12.5 nm (APC)
<b>Optical alignment</b>	Fixed alignment, no maintenance required
<b>Flow cell</b>	200 micron ID fused silica capillary
<b>Minimum particle size</b>	1 µm
<b>Maximum particle size</b>	40 µm
<b>Minimum sample size</b>	30 µL
<b>Nominal flow rate</b>	Slow (14 µL/min), Medium (35 µL/min), Fast (66 µL/min)
	User selectable from 10 to 100 µL/min (CFlow Plus only)
<b>Core diameter</b>	Slow (10 microns), Medium (16 microns), Fast (22 microns)
	User selectable from 5 to 40 microns (CFlow Plus only)
<b>Florescence sensitivity</b>	< 750 MESF FITC
<b>Scatter resolution</b>	Resolves human peripheral blood erythrocytes, lymphocytes, monocytes, and granulocytes
<b>Fluorescence linearity</b>	2.00±0.05% for chick erythrocyte nuclei (CEN)
<b>Fluorescence precision</b>	< 3.0% CV for CEN
<b>Max. event rate</b>	10,000 events/second
<b>Unit volume calculations</b>	CFlow Plus only
<b>Warm-up time</b>	Less than 5 minutes
<b>Power requirements, including cytometer, computer &amp; monitor</b>	AC input 100 - 240V, 50-60 Hz, 750W
<b>Operating ranges</b>	15-30°C; < 80% relative humidity
<b>Instrument size</b>	11.0"H x 14.3"W x 16.5"D (27.9 x 36.3 x 41.9 cm) 27"H x 14.8"W x 17.0"D (68.6 x 37.6 x 43.2 cm) with cover fully open Fluidics bottle tray 10"H x 13.5"W x 4.5"D (25.4 x 34.2 x 11.4 cm)
<b>Weight</b>	< 30 lbs (13.6 kg)
<b>Fluid tank capacity</b>	1L sheath fluid, 1L waste 250 mL cleaner, 250 mL decontamination
<b>Signal processing</b>	24-bit A/D conversion
<b>Computer</b> (minimum specs.)	2.8 GHz Pentium D processor 2 GB RAM 17" Flat-panel monitor
<b>Computer interface</b>	USB 2.0
<b>Software</b>	CFlow or CFlow Plus Software

## 4. SETTING UP THE C6 AND CSAMPLER™

Complete this section (4.1- 4.11) in its entirety before running any samples.

Reference to CFlow refers to common elements in CFlow and CFlow Plus, unless otherwise indicated.

### 4.1. C6 Flow Cytometer Layout

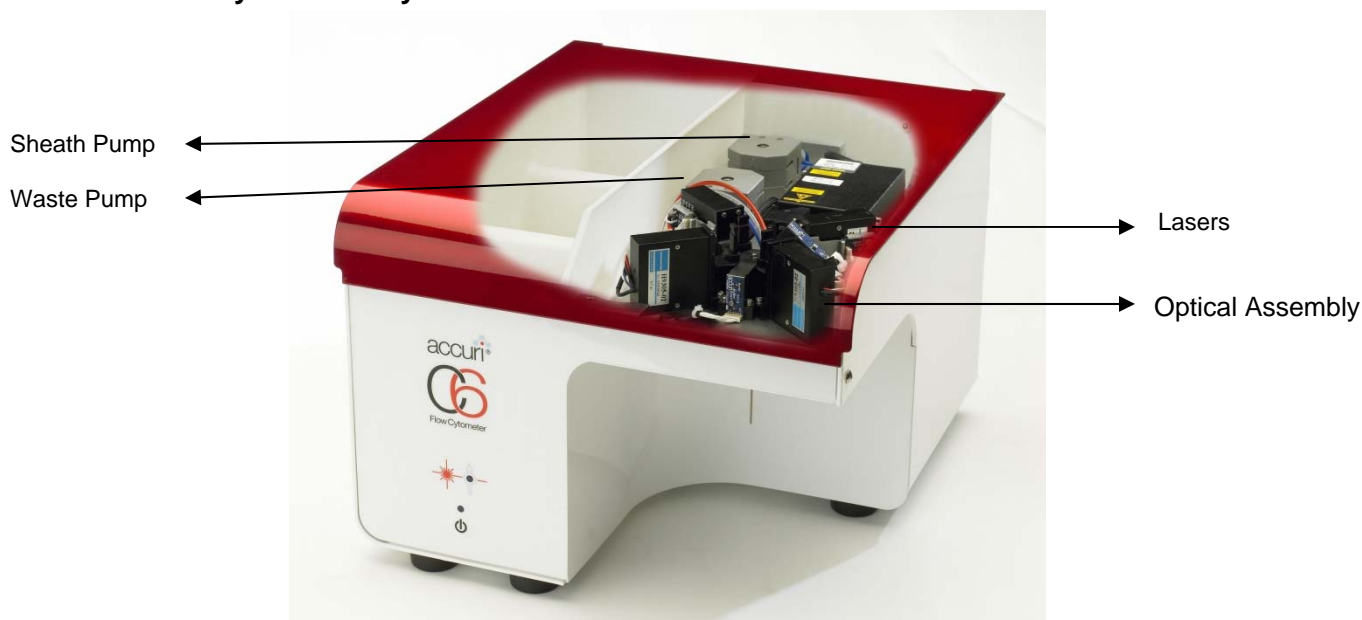


Figure 2. C6 Flow Cytometer Optics and Fluidics

### 4.2. Inspection and Assembly

1. Assemble computer, monitor, keyboard, and mouse according to the Dell Instruction Card.
  - i. **CAUTION** Do not connect the USB cable from the computer to the cytometer yet. Wait until the cytometer is set up and the software is installed to connect the USB cable. (Section 4.8)
  - ii. The computer may not have the proper screen resolution set for optimal display of CFlow Software. To check this setting, right click on the desktop. Select **Properties**. A window entitled Display Properties will open; select the **Settings** tab. Be sure that the Screen Resolution is set to at least 1152 by 864 pixels and that the Color quality is set to Highest (32 bit).
  - iii. The computer may be set to hide file extensions. In order to more easily distinguish CFlow files (.c6) from CFlow Template files (.c6t), you may want to turn off the hidden file extensions. To do this, click on Windows **Start**, then select **My Computer**. Click **Tools** and then **Folder Options** from the pull down menu. Click on the **View** tab, then scroll to **Hidden files and folders** and uncheck the box next to **Hide extensions for known file types**. Then select **OK**.
2. Remove the C6 Flow Cytometer and other components from the shipping boxes. Store the shipping boxes and packaging material in a dry location so they can be used to return the cytometer to Accuri for service, if needed.

3. With the front of the cytometer facing toward, lift the red top cover up by pulling it gently upwards.
4. Verify that the in-line sheath filter is installed. This is the long cylindrical fluid filter with blue fluidic lines running to it (Figure 2). If the in-line sheath filter is not installed, see Section 5.6.
5. Verify that all ribbon cables are connected.
6. Verify that the interference filters are in place. The interference filters are labeled. Interference filters are FL1 - 530/30, FL2 - 585/40, FL3 - 670LP and FL4 - 675/25 (Figure 2).

**CAUTION** Never run the C6 without an interference filter in front of the PMTs, as this may damage the PMTs.

7. Close the cover by gently pushing it downwards. Make sure the cover is securely positioned all the way down. If the cover is not closed securely, the cytometer will not function.
8. Place the fluidics bottle tray in a convenient location near the perimeter of the C6 Flow Cytometer.

**CAUTION** Do not place the fluidics bottle tray on top of the cytometer.

### 4.3. Reagent Bottles and Fluidics

1. Fill Sheath Bottle with Sheath fluid.

Accuri has found no advantage in using commercially available sheath fluids except with cells or particles less than 1µm. In all cases when sheath is referenced, the fluid is HPLC reagent grade filtered, deionized, H<sub>2</sub>O (0.22µm filter), or equivalent, with Bacteriostatic Concentrate Solution (Part# KR-220) added, not saline sheath.

2. Dilute the Decontamination Concentrate (Part# KR-200) and Cleaning Concentrate (Part# KR-210) solutions as stated on the bottles and fill Decontamination and Cleaner Bottles respectively.
3. Check that the Waste Bottle is empty.

**CAUTION** Close bottles securely. Do not over tighten. The C6 Flow Cytometer is a non-pressurized system.

4. Place bottles in fluidics bottle tray.
5. Move the red switch on the black end of the fluidics harness to the unlock position, attach the black end of the fluidics harness to the back of the cytometer in the correct orientation, then lock the red switch.
6. Attach the other end of the fluidics harness to the appropriate bottles; red line to waste, blue line to sheath, yellow line to decontamination and green line to cleaner (Figure 3). Snap the quick disconnects into place.



**Figure 3. Proper Attachment of Fluidics Lines**

#### 4.4. Emptying the Waste Container



Biological samples are potentially dangerous, infectious, and/or life threatening. Proper handling procedures for samples and reagents should be adhered to at all times.

Always treat waste with 0.5% NaOCl before disposal. Dispose of waste according to local regulations.

Always empty the waste tank daily or when prompted by CFlow software to prevent spillover and possible biological safety risk.

To empty the Waste Container:

1. Disconnect the waste tank level sensor and quick disconnect from the top of the bottle.
2. Remove the bottle lid.
3. Dispose of waste.
4. Add approximately 100 mL of 0.5% NaOCl to the Waste Bottle.
5. Replace the bottle lid.
6. Reattach the waste level sensor and quick disconnect to the top of the bottle.

## 4.5. Electrical Connections

1. Attach the black power cord to the power supply and plug into a standard outlet. This instrument should be operated from a supply source which incorporates a third wire protective grounding conductor. A surge suppressor or power strip is recommended. Plug the cord from the power supply into the back of the cytometer (Figure 4).
2. Connect the USB cable to the back of the C6 Flow Cytometer.

**CAUTION** *Do not plug the USB cable into the computer yet. CFlow Software must be installed and open on the computer before connecting the USB cable to the computer for the first time.*



**Figure 4.** Proper Placement of USB cable and Power Cord

**CAUTION** Do not place the C6 against a wall or other object so as to put pressure on the fluidics lines or USB cable connection.

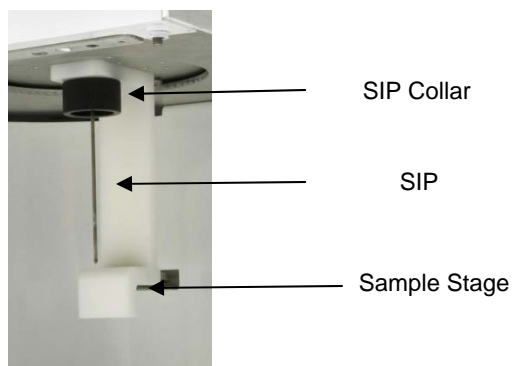
## 4.6. Starting up the C6 Flow Cytometer for the First Time

1. The sample tube holder is located on the front right corner of the C6 Flow Cytometer. Gently push it back and place a 12x75mm tube containing 2 mL of sheath fluid on the Sample Introduction Probe (SIP) (Figure 5).

The sample stage can accommodate several types of tubes, including any brand of 12x75mm tube and most microcentrifuge tubes (with caps removed).

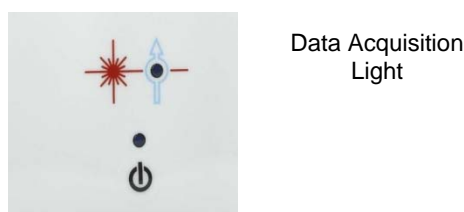
2. While holding the sample tube in place, pull the sample stage forward so that it supports the tube. The stage will lock into place when pulled all the way forward.

**CAUTION** The sample tube does not form a seal at the top of the SIP when inserted. While inserting the sample tube, be careful not to bend or catch the SIP on the outside of the tube.



**Figure 5.** SIP and Sample Stage

3. Turn on the cytometer by pressing the silver button below the data acquisition light once (Figure 6). The button will light up when you press it. The light in the center of the button will flash and then stay lit when the C6 is on.



**Figure 6.** Data Acquisition Light

4. Wait approximately one minute for the pumps to start.
5. When powered on, the C6 will automatically flush the fluidics lines with sheath prior to reporting in the CFlow message box that it is ready for a sample. This process takes less than 5 minutes.

## 4.7. CFlow Software

Windows® XP users, start with step 4.8.a. Windows Vista users, skip to step 4.9.

### 4.7.a. Installing CFlow for Windows XP Users

1. Turn on the computer and monitor.
2. Check to see that the USB cable is not connected between the computer and cytometer.
3. Insert the CFlow Software CD provided by Accuri. Double-click on the program called **CFlowInstaller.exe**. Follow the installation instructions in the Wizard.

### 4.7.b. C6 Driver Installation

1. Double-click the CFlow or CFlow Plus icon on the desktop. An Accuri splash screen will appear for approximately 30 seconds. A CFlow workspace will then open with Plot 1 as a FSC-A vs. SSC-A density plot and several other unassigned plot windows. You can modify Plot 1 and/or

select the other plot windows to be histograms, dot plots, or density plots with the desired parameters. Please refer to the CFlow User's Guide or Collection Quick Start Guide for further directions on using CFlow and CFlow Plus.

2. Connect the USB cable from the C6 cytometer to one of the computer USB ports. Mark the port in case the USB cable becomes disconnected.

**CAUTION** Do not plug the USB cable for the cytometer into the USB ports that are on the computer monitor.

3. If this is the first time you have connected the C6 to your computer, the Hardware Installation Wizard dialog box will appear (Figure 7).
4. Select the option **No, not at this time**, and then click **Next**.

You may or may not see this pop up screen. If not, proceed to Step 5.



**Figure 7.** Hardware Installation Wizard, First Dialog Box

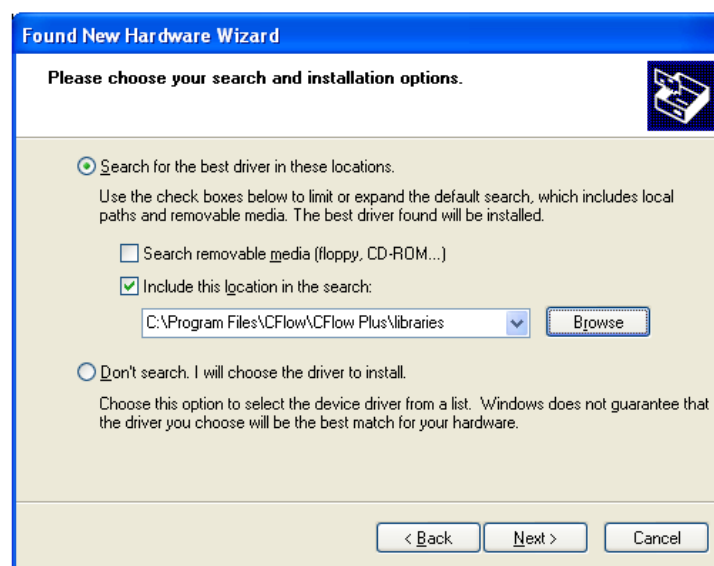
5. When the second dialog box appears, choose the option **Install from a list or specific location (Advanced)**, and click **Next** (Figure 8).



**Figure 8.** Hardware Installation Wizard, Second Dialog Box

6. Specify one of the following paths using the **Browse** button (Figure 9):

**C:\ProgramFiles\CFlow\CFlow\libraries**  
or  
**C:\ProgramFiles\CFlow\CFlowPlus\libraries**



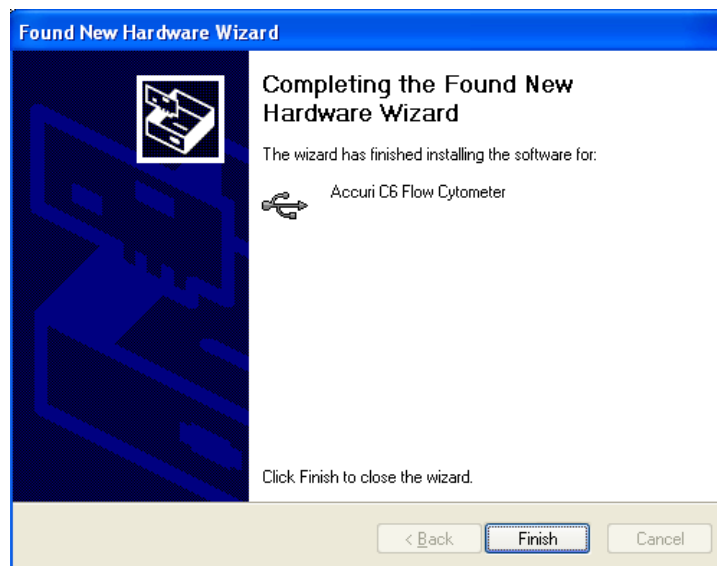
**Figure 9.** Hardware Installation Wizard, File Pathway Designation

7. When the dialog below opens (Figure 10), click **Continue Anyway**.



**Figure 10.** Hardware Installation Wizard Dialog Box

8. When the final dialog box appears (Figure 11), select **Finish**.



**Figure 11.** Final Hardware Installation Wizard Dialog Box

## 4.8. Installing CFlow for Windows Vista users

### 4.8.a. Installing Software

1. Turn on the computer and monitor.
2. Ensure the USB cable is **not** connected between the computer and cytometer.
3. Insert the CFlow Software CD provided by Accuri. Double-click on the program called **CFlowInstaller.exe**. Follow the installation instructions in the Wizard.

### 4.8.b. C6 Driver Installation for Windows Vista Users

1. Double-click the CFlow or CFlow Plus icon on the desktop. An Accuri splash screen will appear for approximately 30 seconds. A CFlow workspace will then open with Plot 1 as a FSC-A vs. SSC-A density plot and several other unassigned plot windows. You can modify Plot 1 and/or select the other plot windows to be histograms, dot plots, or density plots with the desired parameters. Please refer to the CFlow User's Guide or Collection Quick Start Guide for further directions on using CFlow and CFlow Plus.
  2. Connect the USB cable from the C6 cytometer to one of the computer USB ports. Mark the port in case the USB cable becomes disconnected.
- CAUTION** Do not plug the USB cable for the cytometer into the USB ports that are on the computer monitor.
3. If this is the first time you have connected the C6 to your computer, the Hardware Installation Wizard dialog box will appear (Figure 12).
  4. Select the option **Locate and install driver software (recommended)**, and double click on it.



Figure 12. Found New Hardware, First Dialog Box

5. When the second dialog box appears, choose the option **I don't have the disc. Show me other options**, and click **Next** (Figure 13).



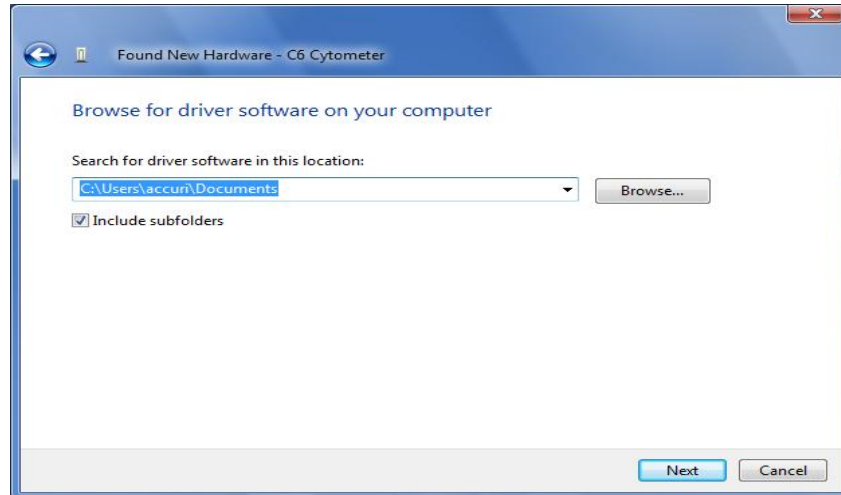
**Figure 13.** Hardware Installation Wizard, Second Dialog Box

- 5.b. When the next dialog box opens (Figure 14), select the option **Browse my computer for driver software (advanced)**.



**Figure 14.** Hardware Installation Wizard, Third Dialog Box

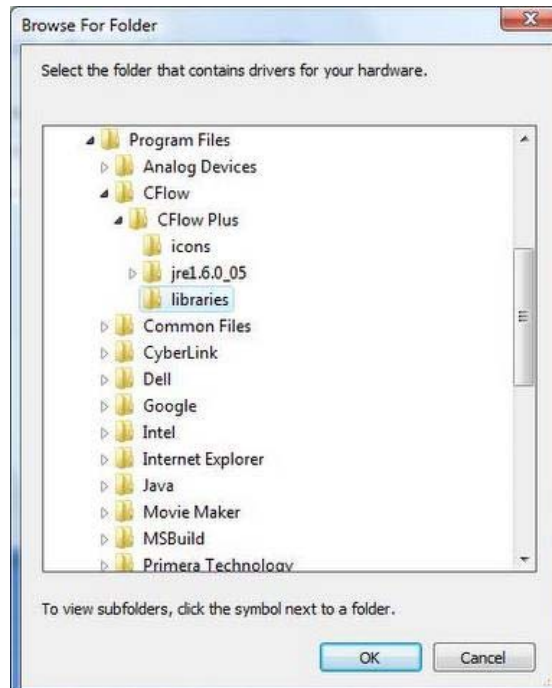
- 5.c. You will be asked to assign the location of the driver software (Figure 15).



**Figure 15.** Hardware Installation Wizard, Fourth Dialog Box

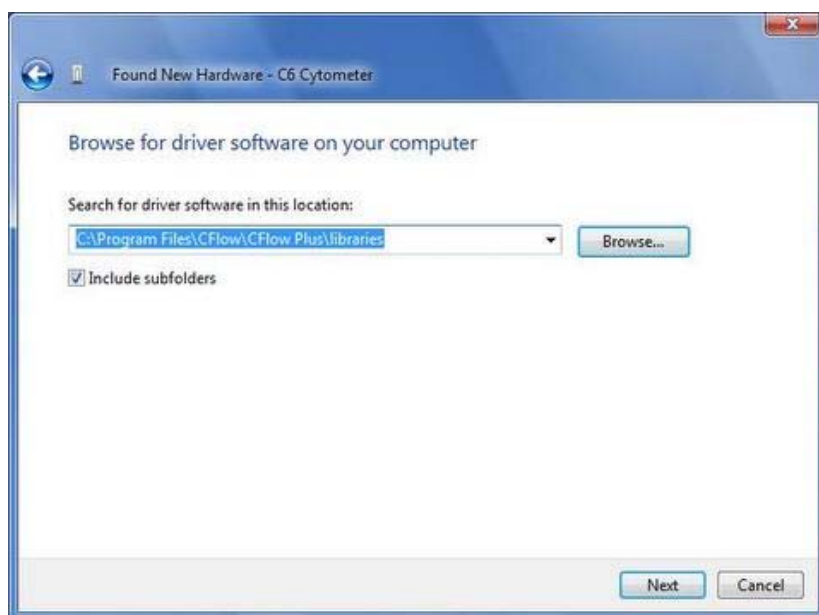
5.d. Specify one of the following paths using the Browse button (Figure 16):

- **C:\ProgramFiles\CFlow\CFlow\libraries, or**
- **C:\ProgramFiles\CFlow\CFlowPlus\libraries**



**Figure 16.** Browse For Folder

5.e. Once driver software is located (Figure 17), select **Next**.



**Figure 17.** Hardware Installation Wizard, File Pathway Designation

- 5.f. When the dialog below opens (Figure 18), a warning will be given that Windows cannot verify the publisher. Select **Install this driver software anyway** and continue.



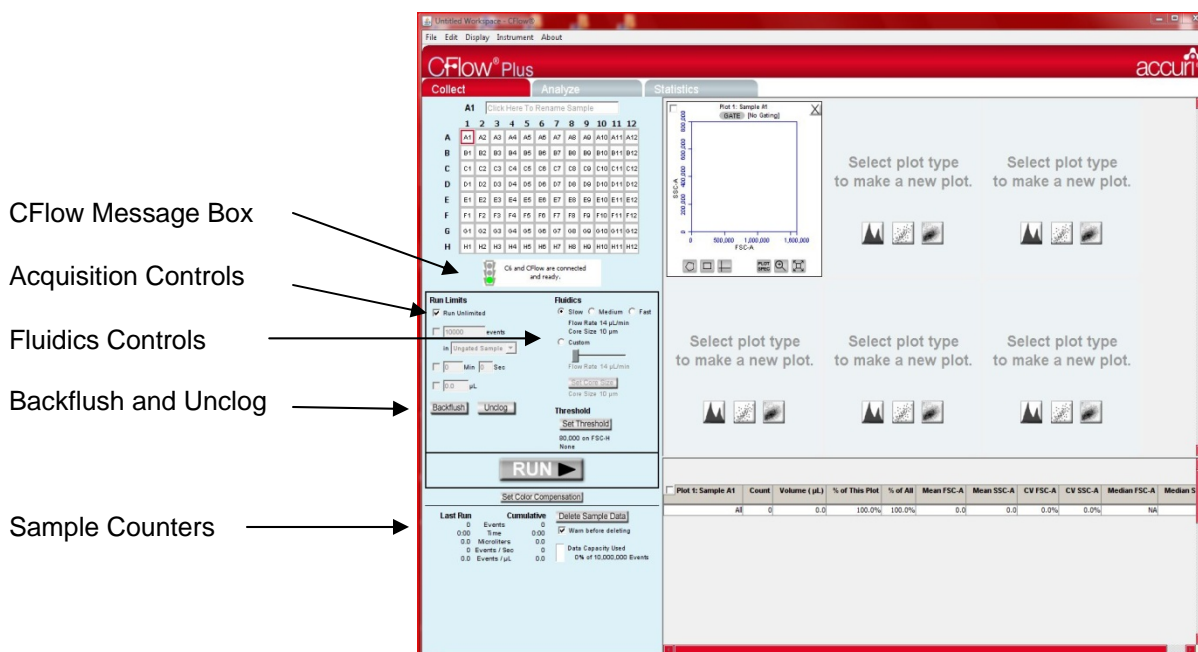
**Figure 18.** Windows Can't Verify The Publisher of This Driver Software

- 5.g. Windows will start the installation of the driver for the C6 Flow Cytometer. Once completed, you have successfully installed the software (Figure 19). Click the **Close** button.



**Figure 19.** The Software For This Device Has Been Successfully Installed.

6. When the USB cable has been properly installed, the CFlow dialogue box will show a green light and the message: **C6 and CFlow are connected and ready**. This indicates recognition of the C6 Flow Cytometer by CFlow software. (Figure 20).



**Figure 20.** CFlow Message Box, Acquisition Controls, Counters, Backflush and Unclog Commands

If the cover of the C6 is not closed securely, the cytometer will not function and CFlow will alert the user, (Figure 21). If the CFlow software issues this error message, push the lid down securely.

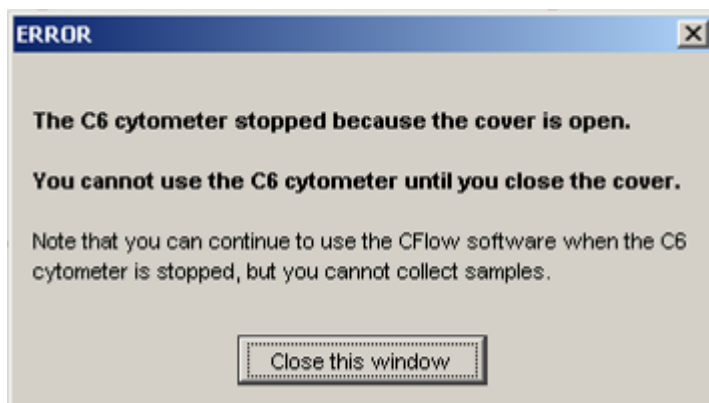


Figure 21. Cover Open Message

## 4.9. Disabling Hibernation & Optimizing Monitor Display Settings

We recommend making the following changes to the C6 workstation computer to achieve the best possible work environment and to avoid unexpected software computer issues that could potentially result in a loss of data.

### 4.9.a Workstation Computer Settings:

1. Go to **Start**, click on the **Control Panel**.
2. From the Control Panel select **Appearance and Themes** and then **Display** or from Windows Classic View select **Display**.
3. Turn off the Hibernation Settings.
  - a. Select the **Screen Saver** Tab and click on the **Power** button (lower left-hand corner).
  - b. From the Power Options Window, change the power settings for Turn off hard disks and System hibernates to the **Never** setting.
  - c. Select **Apply**. Then select **OK**.
4. Optimize Monitor Display Settings
  - a. From **Start**, click on the **Control Panel** and then choose **Display**.
  - b. Select the **Settings** tab. Select **Monitor 1**.
  - c. Ensure that the Screen resolution is set to the maximum setting or to at least 1152 x 864. Change the setting using the slider under Screen resolution, if necessary.
  - d. Check that the **Color quality** setting is set to **Highest (32 bit)**, change the setting using the drop down menu, if necessary.

- e. Click **Apply**.
- f. Select **OK**, and then close the Control Panel.

#### 4.10. Purging Air from the C6 Flow Cytometer

This procedure should be performed if:

- The C6 Cytometer is being set up for the first time, or
- The instrument has sat unused for more than 2 days, or
- You suspect that air may have been introduced into your system by running a sample dry or allowing the instrument to run without sheath in the sheath tank.

In order to purge air:

1. Place a tube containing sheath on the SIP.
2. Select the **Run cleaning fluidic cycle** from the **Instrument** menu in CFlow. This will take approximately 16 minutes to complete.

If you are doing an air purge at any time other than the initial setting up of the cytometer, you may skip step 2.

3. Set the run time in the CFlow control panel for 5 minutes and click "Run". Allow the cytometer to run until the auto stop. The peristaltic pumps will make a rhythmic sound while running. This is normal.
4. Click **Run** again, wait for 30 seconds and then click Pause. The peristaltic pumps will stop.
5. Repeat Step 3 twice more to purge bubbles from the system.

#### 4.11. Validating Performance of the C6 Flow Cytometer

See the CFlow User's Guide (The Basics) for details on instrument validation using the validation beads provided with the cytometer.

#### 4.12. Calibrating the Fluidics of the C6 Flow Cytometer for Precise Volume Measurements

The C6 Flow Cytometer's fluidics system is calibrated for a sample volume of 400  $\mu\text{L}$  in a standard 12x75mm FACS tube during manufacture. It is suggested that you recalibrate your system for the most common tube type and volume when it is received. The C6 can be recalibrated for a specific sample volume or tube at any time. This will increase the accuracy of the volume measurements. Please note, volume measurements can be performed in CFlow Plus software only.

To ensure the most accurate volume measurements:

- The peristaltic pump tubing must be less than two months old.
- Samples should be run at Medium or Fast speeds.
- The C6 should be calibrated to an equivalent sample volume/fluid height in tube as sample to be measured.
- Calibration should never be performed with less than 150  $\mu\text{L}$  in any type of sample tube.

To calibrate the C6:

1. Place a tube of the same kind to be used in your experiment and containing a volume of sheath fluid close to the sample volume in your experiment + 65  $\mu\text{L}$  on the SIP. For example, if your cell samples are diluted to 400  $\mu\text{L}$  in 12x75mm tubes, then the Fluidics Calibration should be performed with a 12x75mm tube containing 465  $\mu\text{L}$  of sheath. This is because Calibration uses ~130  $\mu\text{L}$  of fluid. The cytometer is calibrated at the average volume over the duration of Calibration.

The minimum sample volume for which you can calibrate a 12x75mm FACS tube is 150  $\mu\text{L}$  (run calibrate with 215  $\mu\text{L}$ ).

**WARNING** Do not calibrate the fluidics in any type of sample tube with less than 150  $\mu\text{L}$  in the sample tube.

2. Under the **Instrument** Menu, choose **Calibrate fluidics**. The fluidics calibration will take approximately 5 minutes.

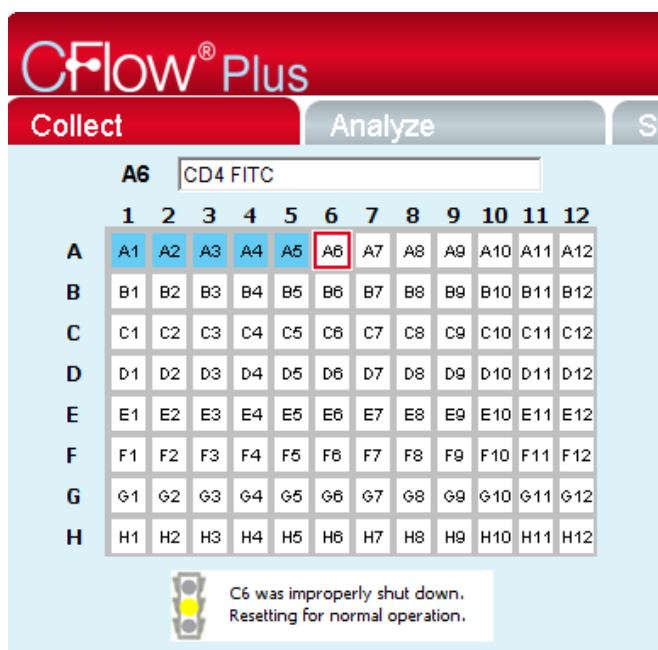
If calibration fails, the cytometer will revert to its previous calibration automatically and the C6 will operate normally. However, the volumes recorded will not be optimized for the new sample volume. If more precise measurement is essential, try calibrating again.

#### 4.13. Turning Off the C6 Flow Cytometer

1. Place a tube with 2mL of 10% Decontamination solution (Part# KR-200) or 0.5% hypochlorite on the SIP.
2. Select an empty data well.
3. Set time limit for 2 minutes and Fluidics speed to **FAST**.
4. Click **RUN**.
5. Once time limit is reached, remove the tube of Decontamination Solution.
6. Place a tube of 2mL filtered, de-ionized  $\text{H}_2\text{O}$  on the SIP
7. Set a time limit of two 2 minutes.
8. Click **RUN**.
9. Leave the tube of  $\text{H}_2\text{O}$  on the SIP.
10. Press the power button once (silver button) or tap the blue touch switch on the front of the cytometer twice within 2 seconds (earlier models). The Decontamination cycle will run for several minutes and then the cytometer will automatically power off. Refer to Section 5.4 to manually decontaminate and clean the cytometer at any time.
11. During the C6 shut down process, the fluidics run automatically, and the tubing and SIP are rinsed with Decontamination solution followed by sheath.

**WARNING** Holding your finger on / depressing the ON/OFF button for 5 seconds or longer will bypass the auto-fluidic shutdown decontamination cycle. If you shutdown the

C6 in this manner, you will see the following message in the CFlow Message Box the next time you power up the C6 (Figure 22):



**Figure 22.** Improper Shut Down of C6 Flow Cytometer

If you see this message, the C6 will take approximately 6 minutes to recover and return to the green light ready state, as opposed to the usual 3 minute startup procedure.

It is not necessary to exit CFlow and to turn off the computer prior to turning off the C6 Flow Cytometer. The computer and CFlow can stay on or off, depending on need.

#### 4.14 Installing the CSampler

The CSampler is an optional accessory for the C6 Flow Cytometer that allows for automated analysis of samples prepared in 48- and 96-well plates and standard 12 x 75 mm tubes in a 24-tube rack.

Install and validate the C6 with 6- and 8-peak beads prior to installing the CSampler. Inspect the Sampler Accessory Kit contents to ensure delivery of all components.



**Figure 23.** CSampler Accessory Kit

**Table 5.** Accuri CSampler Shipping Contents

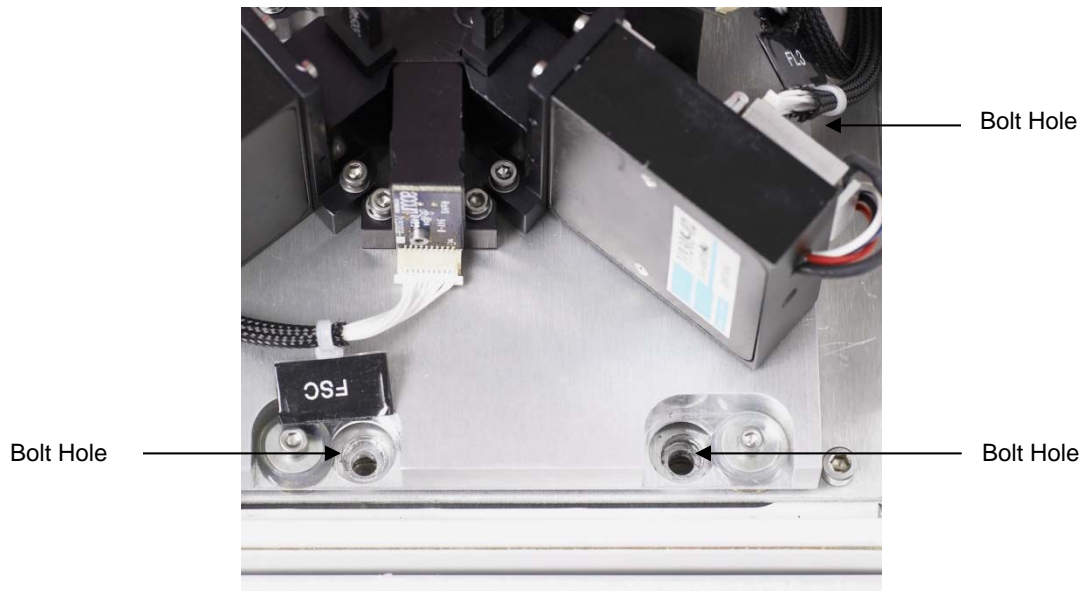
Box 1
<ul style="list-style-type: none"> <li>• CSampler Module</li> <li>• CSampler Mat</li> <li>• Mounting Bolts (3)</li> <li>• Installation and Removal Tool</li> <li>• CSampler Collar</li> <li>• CFlow Sampler Software</li> </ul>

1. Shut down and power off the C6.
2. Unscrew the SIP collar, remove the sample stage and install the CSampler collar (Figure 24).



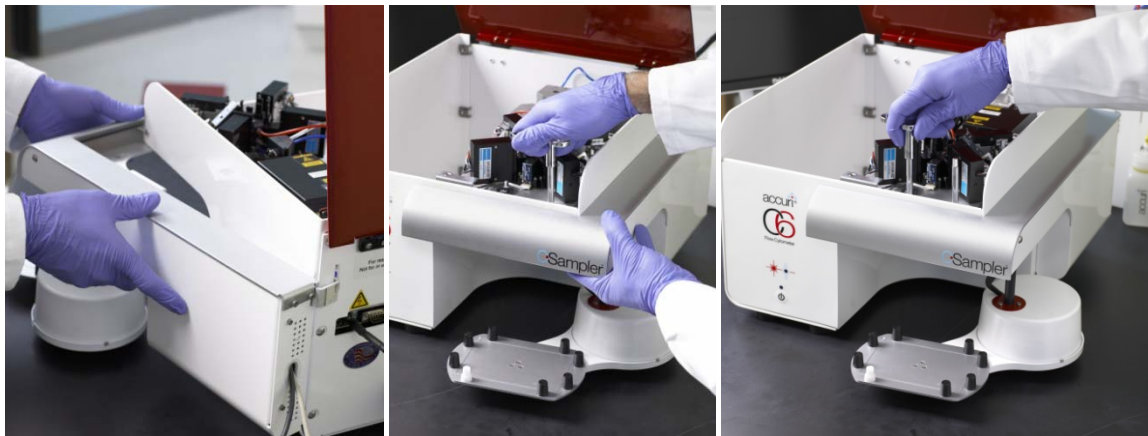
**Figure 24.** Installing the CSampler collar.

3. Open the lid of the C6 and locate the bolt holes used to mount the CSampler (Figure 25).



**Figure 25.** Location of mounting bolt holes

4. Hold the CSampler module with both hands and locate the tab in the slot on the back of the C6 (Figure 26).
5. Align the front of the CSampler so that the holes on the module are lined up with the three threaded holes in the C6. Hold the module with one hand and screw in the right mounting bolt to secure the CSampler to the C6, tighten with the installation and removal tool. Screw in the other two mounting bolts. (Figure 26).



**Figure 26.** Securing the CSampler to the C6.

6. Connect the cable to the socket at the back of the C6 and switch on the C6 (Figure 27). The CSampler will automatically align itself and then remain in the home position.

**CAUTION** Failure to shut down the C6 during CSampler installation could result in damage to both C6 and CSampler electronic modules.



**Figure 27.** Connecting the CSampler cable to the C6.

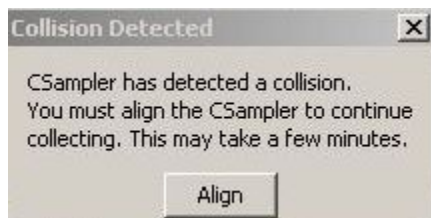
7. Place the mat in position (Figure 28). **CAUTION** Failure to keep the mat clear of plates, tubes or any other materials may result in damage to the CSampler.
8. Install CFlow Sampler software.
9. Run validation beads to ensure correct operation. Refer to Section 4.12.



**Figure 28.** CSampler in home position with mat installed.

## Collisions and Alignment

If there is an obstruction in the path of the CSampler arm, CFlow Sampler will indicate that a collision has occurred. The message box shown in Figure 29 will be displayed, together with a red Traffic Light. In order to continue using the CSampler, an alignment must be performed. Before running an alignment any objects previously placed on the CSampler mat should be removed. Refer to the CFlow User Guide.



**Figure 29.** Collision detected window.

If a second collision occurs a second alignment will begin automatically. If the secondary alignment fails, please contact Accuri Technical Support for further assistance.

## 5. MAINTENANCE



Accuri recommends changing the in-line sheath filter, fluidic tank filters, and peristaltic pump tubing every 2 months. Heavy use of the C6 may warrant more frequent changes. See Section 6.6 or [www.AccuriCytometers.com](http://www.AccuriCytometers.com) for part numbers.

### WARNING

Before performing any maintenance, remove all biological samples from the SIP, run sheath for a minimum of 30 seconds, and turn off the C6 Flow Cytometer. This will ensure that the cytometer's fluidics system has been decontaminated. Biological samples are potentially dangerous and/or life threatening. Proper handling procedures for samples and reagents should be adhered to at all times.

### 5.1. Backflush and Unclog

#### Perform the following procedures if:

During sample accumulation, the C6 event rate drops or goes to zero, possibly indicating a clog in the SIP or flow cell,

**OR**

Validation bead performance does not meet specifications, as detailed in the CFlow User's Guide, possibly indicating air bubbles in the fluidics lines or flow cell.

1. Push the sample tube holder back and carefully remove the sample tube from the SIP.

Be sure to remove sample tube before clicking on Unclog or Backflush, as Sheath fluid will drip into tube possibly causing dilution.

2. Place a container or absorbent material (paper towels) on the bench top under the SIP. Click on the Backflush command in CFlow. Normally, fluid should drip or stream from the SIP during Backflush.
3. If no fluid drips from the SIP during Backflush or if one is trying to purge air bubbles from the fluidics lines, click on the Unclog command. (The Unclog command clears the flow cell with a high volume of sheath fluid at a high velocity.) Repeat Unclog at least once.
4. If after 2 or more Unclog cycles there is still no fluid exiting the SIP upon Backflush, place a tube containing 2 mL of 0.5% NaOCl on the SIP, move to an empty well in the CFlow template and allow the 0.5% NaOCl to be aspirated for 5 minutes.
5. Repeat Unclog and Backflush. If still no fluid exits SIP, the SIP or flow cell may need to be replaced. Call Accuri Technical Support at 1.734.994.8000.
6. Once the clog or air bubbles are cleared, run the fluorescent validation beads to check performance.

## 5.2. Emptying the Waste Container



1. There are sensors in each fluidics bottle which will warn the user through messages in CFlow when bottles need attention.
2. These messages should be responded to in order to ensure proper function of the C6 and prevent possible biohazard risks.
3. The reagent bottles should be visually checked every time the C6 is used.

Biological samples are potentially dangerous and/or life threatening. Proper handling procedures for samples and reagents should be adhered to at all times. Wear appropriate laboratory attire such as gloves during this procedure.

Always empty the waste tank daily or when prompted by CFlow software to prevent spillover and possible biological safety risk.

1. Disconnect the waste level sensor and quick disconnect from the top of the Waste Bottle.
2. Remove the lid.
3. Dispose of waste according to local regulations.
4. Add approximately 100 mL of 0.5% NaOCl to the bottle.
5. Replace the lid.
6. Reattach the waste level sensor and quick disconnect to the top of the bottle.

## 5.3. Filling the Sheath, Decontamination, and Cleaning Solution Bottles

Before turning on the C6 Flow Cytometer, visually check all the bottles and fill the Sheath, Decontamination, and Cleaning Solution Bottles accordingly.

1. Disconnect the appropriate level sensor and quick disconnect from the top of the bottle.
2. Remove the bottle lid.
3. Fill the bottle with appropriate reagent.
4. Replace the lid.
5. Reattach the level sensor and quick disconnect to the top of the bottle.

The C6 Flow Cytometer is a non-pressurized system. If needed, any of the bottles can be opened while the C6 is powered on.

The fluid sensors will alert the operator when the fluid bottles need attention or if a sensor is improperly connected. The bottles should be visually checked every time the cytometer is used.

## 5.4. Decontamination and Cleaning Cycles

### 5.4.a. Running a Manual Decontamination Cycle

Although CFlow will automatically run a Decontamination Cycle during shut down, this cycle can be run manually at any time to decontaminate the cytometer.

1. Remove the sample tube and place a tube of Decontamination Solution or 0.5% NaOCl on the SIP.

Do not use household bleach as it contains fluorescent whitening agents which may interfere with the fluorescence of your samples.

2. Select **Run Decontamination Fluidic Cycle** from the CFlow Instrument menu in the command line to start the cycle. This cycle takes approximately 5 minutes.
3. After the decontamination cycle is complete, replace the tube of cleaning fluid with a 12x75m tube of sheath. Run for at least 1 minute.

### 5.4.b. Running the Cleaning Cycle

Accuri recommends running the cleaning cycle at least once per week to keep the SIP and flow cell clean.

1. Place either a tube Cleaning Solution on the SIP. If using cleaning fluid, dilute as directed on the cleaning fluid packaging.
2. Select **Run Cleaning Fluid Cycle** from the CFlow Instrument menu to start. This cycle will take approximately 15 minutes.
3. After the cleaning fluidic cycle is complete, replace the tube of cleaning fluid with a 12x75mm tube of water. Run for at least 1 minute.

## 5.5. Inspection of the Fluidic Lines



Accuri recommends that the cytometer is checked periodically to ensure there are no fluid leaks.

To inspect the instrument:

1. Turn off the cytometer.
2. Open the top cover.
3. Visually inspect that there are no fluid leaks by looking for small pools of liquid near any of the visible luer lock connects.
4. Visually inspect for dried residue or slight discoloration of the metal fluidics tray plate.

If any evidence of a leak is seen, Accuri Technical Support should be contacted immediately. No attempt should be made to repair the instrument.

Any evidence of fluid coming from, or near, a red or clear line should be considered a biological hazard. Do not attempt to clean up the fluid without gloves and other safety attire. Dispose of all cleaning materials as if biologically contaminated.

## 5.6. Replacing the In-line Sheath Filter



Accuri recommends replacing this filter every two months (Part# CP-140). If the C6 is used daily for more than a few hours, Accuri recommends replacing the filter monthly. Wear appropriate safety attire such as protective gloves and eyewear when performing this action.

1. Turn off and unplug the C6 Flow Cytometer.



**Figure 30.** The In-line Sheath Filter

2. **Open the cytometer** cover by lifting the lid gently upwards.

3. Disconnect the luer locks on the outside of the in-line sheath filter by unscrewing (Figure 30) and discard the filter. Although sample material does not pass through this filter, it is recommended that it should be discarded in the same way as any biological sample to ensure no possible biohazard safety risk.

4. Replace with a new, vented in-line sheath filter (Part# CP-140). This filter has a male and female end so it can only be installed in the correct orientation.

5. Reconnect the luer lock fittings to the filter.
6. Close the cytometer cover by gently pushing the lid downwards until closed.
7. Place a sample tube with sheath on the SIP. Plug in and turn on the cytometer. Purge any air from the instrument. (Section 4.11.)



## 5.7. Replacing the Fluidic Bottle Filters

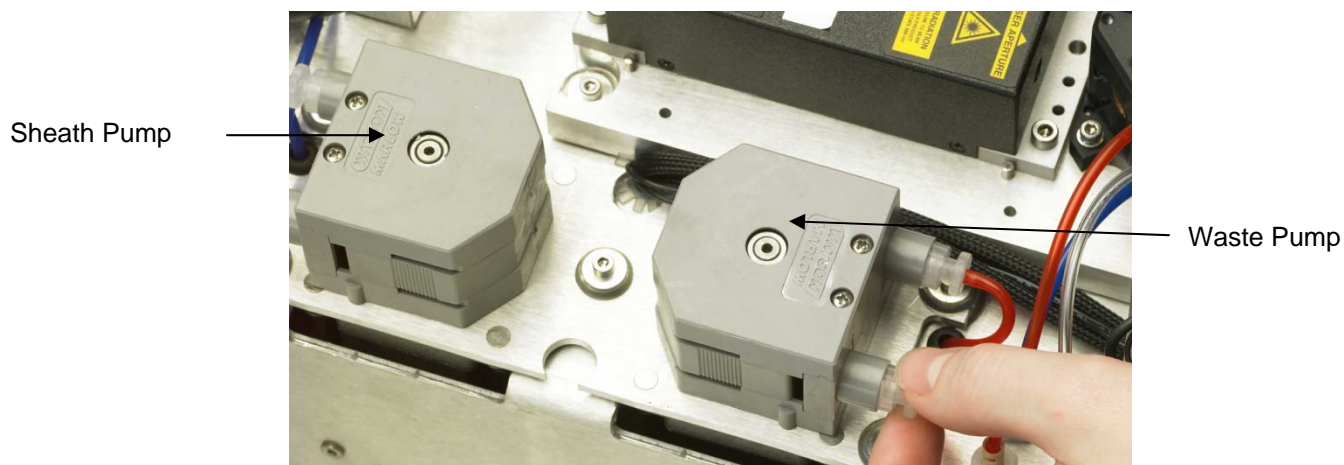
In each of the Sheath, Cleaner, and Decontamination tanks, there is a disk filter. Accuri recommends replacing these filters every two months (Part# CP-130 and CP-135). Wear appropriate safety attire such as protective gloves and eyewear when performing this action.

1. Disconnect the quick disconnect and level sensor connections from the top of each bottle.
2. Carefully remove the lid and level sensor assembly from each bottle.
3. Unscrew the luer lock filter at the end of the fluidic tubing and discard it. Accuri recommends discarding of these filters as you would any biological sample to prevent any possible biological safety risk.
4. Replace the filter with its appropriate replacement. (Sheath Bottle = Part# CP-130, large disk filter; Cleaner and Decon Bottles = Part# CP-135, small disk filter).
5. Re-assemble bottles and reconnect level sensors and fluidics.
6. Place a tube of H<sub>2</sub>O on the SIP and run for 1 minute.

## 5.8. Replacing the Peristaltic Pump Tubing



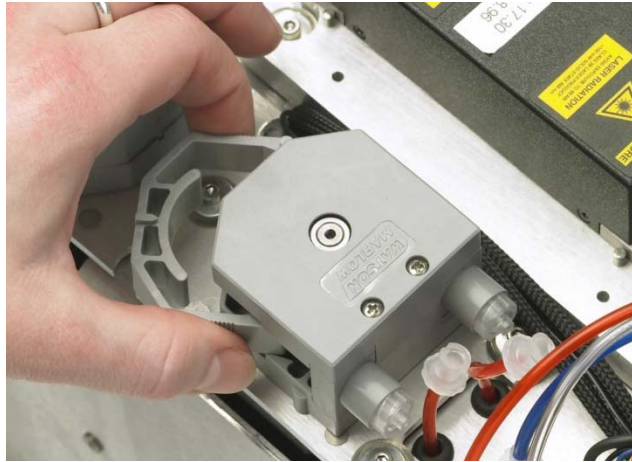
This procedure should be done every two months (Part# CP-105). It is recommended that both pieces of tubing are replaced at the same time. Wear gloves and appropriate safety gear during this procedure as this tubing comes in contact with biological samples and therefore should be considered hazardous. There are two peristaltic pumps, a sheath pump and a waste pump (Figure 31).



**Figure 31.** Peristaltic Pumps and Luer Locks

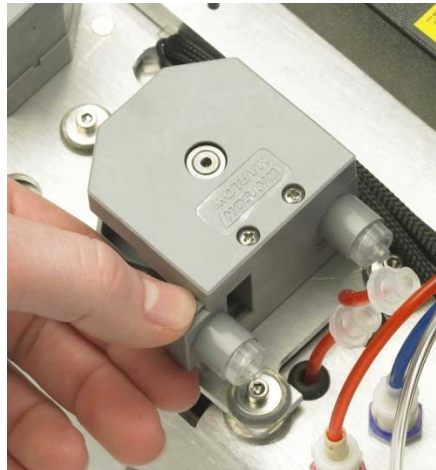
1. Turn off and unplug the C6 Flow Cytometer.
2. Open cytometer by lifting the cover upwards.
3. Disconnect the luer locks to the tubing on the outside of the peristaltic pumps by unscrewing them (Figure 31). Mark the tubing on the outside of the peristaltic pump, if desired, to ensure correct re-assembly. Blue tubing is connected to the sheath pump and red tubing to the waste pump.

4. Squeeze the grip marks on the pump element retainer clip to remove the clip (Figure 32).



**Figure 32.** Removing Peristaltic Pump Retainer Clip

5. Pull the luer lock connectors outward and slide the luer lock fittings off the pump head (Figure 33).



**Figure 33.** Sliding Luer Lock Fittings Off of Pump Head

6. Remove the peristaltic pump tubing and discard it as you would a biological sample according to standard laboratory protocols and regulations. This pump tubing should be considered biologically hazardous as sample passes through it.
7. Replace with new peristaltic pump tubing (Part# CP-105) by sliding the luer lock fittings on the pump head. Snap in place. To facilitate installation, attach the interior luer locks, closest to other pump, first.
8. Replace the pump element retainer clip. Reconnect the luer lock fittings on the outside of the pumps.

**CAUTION** Ensure the luer lock fittings are connected to the correct tubing elements. When re-attaching the peristaltic tubing connectors to the cytometer fluidic lines, the left fluidic line coming from the cytometer in front of each pump should attach to the left end of peristaltic tubing on each pump.

9. Close the C6 cytometer cover by gently pushing the lid downwards.
10. Place a sample tube with sheath on the SIP.
11. Plug in and turn on the cytometer.
12. Purge air from the cytometer. (Refer to Section 4.10)

## 6. TROUBLESHOOTING AND TECHNICAL SUPPORT

### 6.1. Troubleshooting

1. **The cytometer and/or computer does not power on.**
  - Check to make sure power supplies and cords are plugged into an appropriate outlet.
  - Check the outlet to make sure it is functioning properly.
2. **The acquisition controls on CFlow do not show a green light and remain a dimmed grey color after the cytometer is powered on and CFlow is loaded.**
  - Check to see that the USB cable is properly attached to the cytometer and computer. It may take several seconds for the port to see the cytometer.
  - Switch the USB cable to a different port on the computer.
  - Check that the C6 cytometer was properly installed, via the computer Hardware Manager. If necessary, reinstall the driver.
  - If this occurred upon initial installation, be sure that the computer was on and CFlow was installed and open **before** the USB cable to the cytometer was connected. The cytometer should also be powered up before connecting the USB cable to the computer.
3. **Data does not look as expected.**
  - Pause and Restart the sample.
  - Run Backflush and/or Unclog (Section 5.1).
  - Run the Accuri validation beads. If the beads look fine, there may be a problem with your sample. If the beads are out of spec, do a manual Decontamination (Section 5.4). Run beads again, if still out of spec, call Accuri Tech Support.
4. **The event rate drops off while running a sample, but the C6 is not clogged.**
  - Place a tube of Cleaning Solution (1x) on the SIP and run for 5 minutes.
  - Run H<sub>2</sub>O for 1 minute.
  - Check to see if the event rate is restored.
5. **The cytometer pumps are running normally, but CFlow is not acquiring data.**
  - Check the levels in Sheath and Waste Bottles.
  - Check to see if any of the external fluidic lines are crimped.
  - Run Backflush to see if the SIP is clogged (Section 5.1).
  - Check that the peristaltic pump tubing and retainer clip are properly attached (Section 5.8).
6. **The C6 Flow Cytometer pumps are running continually.**
  - Check for a large air bubble in the in-line sheath filter. If so, replace the filter (Section 5.6).
  - There could be a clog in the line. Run Backflush or Unclog.
  - The peristaltic pump tubing may need to be replaced.

For additional technical support, view Frequently Asked Questions (FAQs) at [www.AccuriCytometers.com/customer\\_support/faqs/](http://www.AccuriCytometers.com/customer_support/faqs/) or contact Accuri Technical Support if a problem persists.

## 6.2. Technical Support

For Technical Support contact:

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<a href="http://www.AccuriCytometers.com">www.AccuriCytometers.com</a>	

## 6.3. Returning the C6 Flow Cytometer to Accuri for Service

A return authorization number and a signed form certifying that the instrument is clean are required for the return of an instrument to Accuri for any reason. Contact Accuri Technical Support for information. Visit [www.AccuriCytometers.com](http://www.AccuriCytometers.com) for information on service contracts.

### 6.3.a. Cleaning and Decontamination - Standard Procedure



This protocol should be followed if the C6 is functional when it is to be returned to Accuri. Take appropriate safety measures such as wearing gloves and proper laboratory attire while performing this procedure.

1. Turn on the C6 Flow Cytometer and open CFlow.
2. Check to see that the Sheath, Cleaning, and Decon Bottles are full and the Waste Bottle is empty.
3. Place a 12x75mm sample tube containing 4 mL of Cleaning Solution on the SIP. Using CFlow, activate the manual Cleaning cycle by selecting the **Run Cleaning Fluid Cycle** command from the CFlow Instrument menu in the command line.
4. After the Cleaning Fluidic Cycle is complete, replace the tube with a 12x75mm tube containing 4 mL of Decontamination fluid and **Run Decontamination Cycle** from the Instrument menu.
5. After the decontamination cycle is complete, uncouple the quick disconnects for the Sheath, Cleaner and Decontamination Bottles on the fluid tanks. **Do not disconnect the Waste line (red).**

**WARNING** When disconnecting the fluidic harness from the C6, always disconnect from the bottle end of the harness first to prevent fluid leaks.

6. Remove in-line sheath filter by disconnecting luer lock connectors from each end (Section 5.6). Reconnect the two blue fluid lines together with the luer lock connectors. Discard fluid filter as you would any biological sample to prevent contamination risk.
7. Remove sample tube from SIP. Place an **empty** 12x75mm sample tube on SIP.
8. Run the **Cleaning Fluid Cycle** from the **Instrument** menu.

The C6 may sound louder and higher-pitched than usual; this is appropriate when drying out the C6 in this manner.

9. Leave the empty tube on the SIP.

10. Turn off the C6.

When the C6 has completed the auto-fluidics cycle and turned itself off, remove sample tube from SIP. There might be fluid in it; this is acceptable. Dispose of fluid as you would a biological sample.

11. Disconnect the Waste line from the Waste Bottle and the fluidics harness from the back of the C6.
12. Wipe down the outside of the C6 with a common laboratory disinfectant such as 70% ethanol or a 0.5% NaOCl solution.
13. Repack the C6 in the original packaging material or the material from the C6 loaner unit. Mark the return authorization number on the front of the shipping box.
14. Fax the signed form certifying that all chemical, biological and radioactive hazards have been removed from the cytometer to Accuri Technical Support at 1.734.994.8002. Accuri will not accept the shipment if the form certifying that the instrument has been decontaminated is not on file.

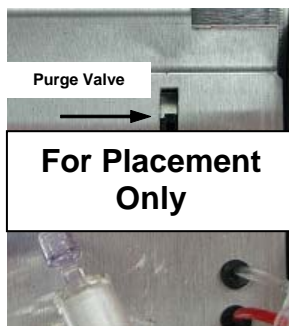
### 6.3.b. Cleaning and Decontamination - Irrecoverable Procedure



This procedure is to be followed if the C6 is unable to operate normally and has run an auto-fluidic shutdown cycle. Wear appropriate safety attire as there may be biological contamination within the instrument.

1. Ensure the C6 is plugged in. The C6 does not need to be turned on, but it must be plugged in for this procedure to work.
2. Remove any sample tube from the SIP and replace with a 12x75mm FACS tube containing 4 mL of diluted Decontamination Solution.
3. Disconnect the fluidic harness lines from the Sheath, Cleaner, Decontamination and Waste Bottles.  
**WARNING** When disconnecting the fluidic harness from the C6, always disconnect from the bottle end of the harness first to prevent fluid leaks.
4. Open the cover and remove the in-line sheath filter (Section 5.6). Connect the two blue fluidic line ends together with the luer locks. Do not install a new filter. Discard the used filter as if biologically contaminated.
5. If the C6 is turned on, turn off the instrument. Disconnect the USB cord and power supply from the back. Remove the 6 Phillips screws in the back cover of the C6 and remove the back cover. A small black switch will be seen, recessed in the fluidics board (Figure 34). This is the Purge Valve Switch.
6. Replug in the power supply but do not turn the C6 on. Re-attach the fluidics harness to the back of the C6 and the Waste Bottle only. Move the Purge Valve Switch to the forward position (Purge). You should hear the valve "click".

7. Disconnect the two luer locks connecting the red tubing to the waste pump. The waste pump is the left pump.



**Figure 34.** Purge Valve Override Switch

8. Attach a clean 10 mL syringe to the luer lock on the red tubing disconnected from the back of the right side of the waste pump.
9. Pull back slowly on the syringe until the 10 mL mark is reached. The tube on the SIP should empty during this procedure. Disconnect the syringe and empty it into a container containing 0.5% NaOCl. Reconnect the syringe to the same luer lock and repeat. During the second syringe pull air should be seen entering the syringe.
10. Move the Purge Valve Switch to the back position (Waste). The valve will give an audible “click”. Refill the tube on the SIP with 4 mL of Decontamination Solution. Empty the syringe. Repeat steps 9 and 10.
11. Fill the syringe with 4 mL of Decontamination Solution (1x). Connect it to the left red line that was connected to the waste pump. Make sure the Waste line and Waste Bottle are still attached to the cytometer otherwise Decontamination Solution will spill out the back of the instrument and onto the bench-top.
12. Slowly inject the contents of the syringe until empty.
13. Refill the syringe with air and reconnect to the left waste pump fluid line. Slowly push the air through the line. This forces all of the Decontamination Solution from the line and into the Waste Bottle.
14. Remove the peristaltic tubing from the waste pump, discard it as a biological hazard, and close the cytometer cover. The two red fluid lines that were connected to the waste pump should remain unattached.
15. Disconnect the waste line from the Waste Bottle and remove the fluidics harness from the back of the C6.
16. Remove the power cord from the C6. Re-attach the back cover.
17. Unscrew the lids from the four bottles, disconnect the fluidics harness and discard liquid, along with the liquid from the fluidics harness, according to proper handling procedures. **Do not** return bottles, fluidics harness and fluidics bottle tray to Accuri.

18. Use the original packaging material or the material from the C6 loaner unit and pack the C6 Flow Cytometer. Mark the return authorization number on the front of the shipping box.
19. Fax the signed form certifying that all biological, chemical, and radiological hazards have been removed from the C6 Flow Cytometer to Accuri Technical Support at 1.734.994.8002. Accuri will not accept the shipment if the form certifying that the instrument has been decontaminated is not on file.

## 6.4. Limited Warranty

Limited Warranty Period is one (1) year from the earlier of, (i) first operation of the Products by Buyer or (ii) ten (10) days after shipment of the Products to Buyer.

This Limited Warranty covers software and firmware, defects in material and workmanship under normal use and does not apply to ordinary wear and tear. For more detailed information, refer to the warranty information at [www.AccuriCytometers.com](http://www.AccuriCytometers.com).

## 6.5. Service Contracts

Service contracts are available. Visit [www.AccuriCytometers.com](http://www.AccuriCytometers.com) for information.

## 6.6. Ordering Information

### 6.6.a. Replacement Parts and Reagents

See [www.AccuriCytometers.com](http://www.AccuriCytometers.com) for a complete list of parts and reagents available from Accuri Cytometers.

**Table 6.** Accuri C6 Replacement Parts/Reagents List

Part Number	Description	Replacement Interval
CP-105	Peristaltic Pump Tubing (Set of 2)	Every 2 Months
CP-130	Sheath Bottle Filters (Large) (Pk of 5)	Every 2 Months
CP-135	Cleaner / Decon Bottle Filters (Small) (Pk of 5)	Every 2 Months
CP-140	In-line Sheath Filters (2 ea.)	Every 2 Months
CP-158	C6 Flow Cytometer Maintenance Kit (1 year supply of Peristaltic Tubing (CP-105), Bottle Filters (CP-130 and CP-135) and In-line Sheath Filters (CP-140))	Sufficient parts for replacement every two months for one year
KR-200	Decontamination Concentrate Solution (10x)	As Needed
KR-210	Cleaning Concentrate Solution (10x)	As Needed
KR-220	Bacteriostatic Concentration Solution (For Sheath Fluid) (10 vials)	As Needed
KR-320	C6 Flow Cytometer Fluid Kit contains concentrate for making 1 L each of Cleaning and Decontamination Solutions and Bacteriostatic Concentrate for treating 10 L of Sheath	As Needed

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